Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae)

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Abstract. In the years 2000 to 2003, 93 *Maculinea alcon* ([Denis & Schiffermüller], 1775) larvae from three localities in Hungary and 261 *M. rebeli* (Hirschke, 1904) larvae from three localities in Hungary and from one locality in Austria were introduced into 103 *Myrmica* colonies in the laboratory. Seven specimens of *M. rebeli* pupated after only about a month in artificial *Myrmica scabrinodis* Nylander, 1846, *My. sabuleti* Meinert, 1860 and *My. salina* Ruzsky, 1905 nests. This phenomenon was found in each of the four studied populations. Two pupae successfully eclosed, one 32 and the other 47 days after adoption. The other five pupae died. These results confirm observations that the developmental time of *M. rebeli* larvae can be plastic. A similarly accelerated development of *M. alcon* larvae was never observed. The accelerated development of *M. rebeli* larvae might be attributed to (1) the higher temperatures in the laboratory as compared with natural conditions, and/or to (2) the artificial *Myrmica* nests which were more exposed to light than under natural conditions, and/or to (3) the balanced artificial diet that the *Myrmica* colonies received.

Key words. *Maculinea, Myrmica,* myrmecophily, host ant, ant diet, accelerated development.

Introduction

Larvae of *Maculinea* van Eecke, 1915 are obligate parasites of *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae) colonies for most of their life. It has long been known that the butterflies have an annual life cycle, with larvae living for about 10–11 months in *Myrmica* ant nests (Thomas 1995; Thomas & Elmes 1993, 2001; Thomas & Wardlaw 1992; Thomas et al. 1989, 1993; Wardlaw et al. 2000). More recently, it has been shown that some larvae live for an additional year in the ant nests, for a total of about 22–23 months (Als et al. 2001; Elmes et al. 2001; Schönrogge et al. 2000; Thomas et al. 1998). While rearing *Maculinea rebeli* (Hirschke, 1904) larvae in the laboratory, an unexpectedly accelerated development was observed, with pupation as soon as a month after adoption.

Although genetic (Als et al. 2004; Bereczki et al. in press) and morphological (Pech et al. 2004) differentiation between the traditionally separated species *M. alcon* ([Denis & Schiffermüller], 1775) and *M. rebeli* is rather low, the two taxa have different physiological and ecological adaptations (see e.g. Schönrogge et al. 2000; Thomas et al. 1989). I use *'M. rebeli'* for populations which develop on *Gentiana cruciata* and *'M. alcon'* for those which develop on *G. pneumonanthe*. However, the host plant affinities of these two taxa need to be re-investigated for their significance for identification purposes (see e.g. Kolev 2002; Munguira & Martin 1999; Sielezniew & Stankiewicz 2004).

Material and Methods

Between 2000 and 2003, 93 *Maculinea alcon* and 261 *M. rebeli* larvae were reared in 103 artificial laboratory colonies of *Myrmica*. For this purpose, plants of

G. pneumonanthe with eggs of *M. alcon* from three localities (Hungary: Fülesd, Gyöngyös, Mátraszentimre) and plants of *G. cruciata* with eggs of *M. rebeli* from four localities (Austria: Hochschwab; Hungary: Bükk-plateau, Bükkszentkereszt, Jósvafő) were collected. In the laboratory the gentians were kept in glasses of water placed in plastic basins, and could be kept fresh for 2–3 weeks while the *Maculinea* larvae emerged. Fourth instar larvae were collected using a fine brush as they dropped from the flowers in the evenings, and were transferred straight into the foraging arena of an artificial *Myrmica* nest to be adopted by the ants. Before introduction, the length of each caterpillar was measured with a ruler. Caterpillars were remeasured after one month by putting the ruler to the glass that covered the artificial nests.

The *Myrmica* colonies usually were collected from the same sites as the gentians. Each colony contained at minimum one queen and 100 workers. They were kept in Debrecen (Hungary) in unheated nests (made from clay and glass) joined by silicon tubes to plastic arenas. These nests were not covered to exclude the light, but were kept in places that never received direct sunlight. The laboratory was not air-conditioned in the summer, but was heated in the colder seasons. The temperature that the *Myrmica* nests experienced was less variable than under natural conditions, and was often up to 25° C in the warmer periods. A part of the nest area was always kept wet by a cotton wool strand that connected the clay with water. To feed the ants, the arenas of the nests were always provided with a cube of sugar, and various insects (mainly cut-up mealworms, larvae and pupae) as well as granules of a dry diet at a minimum of once a week (see appendix). The cube of sugar provided continuous food while the dry diet provided the proteins (and maybe essential vitamins and minerals) when there were not enough insects to feed the ant colonies.

The following *Myrmica* species (identified by Tartally & Csősz) were used: *My. lonae* Finzi, 1926 (1 culture); *My. vandeli* Bondroit, 1920 (1 c.); *My. rugulosa* Nylander, 1849 (1 c.); *My. salina* Ruzsky, 1905 (3 c.); *My. specioides* Bondroit, 1918 (3 c.); *My. gallienii* Bondroit, 1919 (7 c.); *My. schencki* Viereck, 1903 (8 c.); *My. ruginodis* Nylander, 1846 (8 c.); *My. rubra* (Linnaeus, 1758) (9 c.); *My. sabuleti* Meinert, 1860 (9 c.) and *My. scabrinodis* Nylander, 1846 (35 c.).

When a butterfly larva pupated, it was removed from the ants using a pair of fine forceps and placed in a plastic box with ventilation holes and a moist sponge pad at the bottom. This was thought to be important because the ants damage the eclosed butterflies if they are not able to escape from a closed artificial nest and if they are not discovered and separated in time (Elfferich 1988). Voucher samples of ants, dead pupae, exuviae, and butterflies are stored in the author's collection.

Results

Several larvae died during the period of adoption and the next few days. After this critical period their mortality was lower and the *M. rebeli* larvae usually grew very quickly. They were about 3 mm long on introduction, and usually they had grown to about 15 mm a month later. However, seven of them pupated after about a month in different ant nests. These were associated with three *Myrmica* species and came from

Tab. 1. The *Maculinea rebeli* larvae that pupated in about a month in the laboratory.

Locality	Host	Date of adoption	Date of pupation	Date of eclosion
Hungary / Bükk-plateau	*My. sabuleti*	27.07.2002	15.08.2002	28.08.2002
Hungary / Bükk-plateau	*My. scabrinodis*	27.07.2002	28.08.2002	died
Hungary / Bükkszentkereszt	*My. scabrinodis*	11.07.2002	13.08.2002	27.08.2002
Hungary / Bükkszentkereszt	*My. scabrinodis*	11.07.2002	13.08.2002	died
Hungary / Jósvafő	*My. salina*	11.07.2002	15.08.2002	died
Hungary / Jósvafő	*My. scabrinodis*	11.07.2002	10.08.2002	died
Austria / Hochschwab	*My. sabuleti*	14.07.2003	16.08.2003	died

each of the four *M. rebeli* populations studied (Tab. 1). Such a quick development in *M. alcon* larvae was never observed in my experiments during the first months. The *M. alcon* larvae also were about 3 mm long on introduction, but they had grown only to ca. 5 mm a month later and remained about this size in the winter. Two male butterflies from the seven pupae emerged. One of them eclosed 32, the other 47 days after adoption as freshly moulted fourth instar larvae (Tab. 1). These specimens were smaller than average (the forewing length of the one from Bükkszentkereszt was 15 mm and the one from Bükk-plateau 15.5 mm), but similarly small specimens often occur under natural conditions. The fast-developing specimens did not show any other obvious differences compared with field-grown specimens. The other five pupae became rotten or dried out under the unnatural air humidity of the laboratory.

Discussion

The fast-pupating larvae were reared by three different species of *Myrmica*: *My. scabrinodis*, *My. sabuleti*, and *My. salina*. According to field observations, the former two are suitable host ants for *M. rebeli* in Hungary (Tartally & Csősz 2004) and *My. sabuleti* is also suitable in Eastern-Austria (Steiner et al. 2003). However, there are no records of *My. salina* as a host of *M. rebeli* yet (Als et al. 2004; Tartally & Csősz 2004). It is important to note that in well-fed laboratory nests the survival of adopted larvae is usually better than in nature (Elmes et al. 2004; Schönrogge et al. 2004).

The fast development of *M. rebeli* under laboratory conditions might be caused by (1) the warmer temperatures in comparison to natural conditions (Wardlaw 1991; Wardlaw et al. 1998), and/or (2) the artificial *Myrmica* nests being more exposed to light than under natural conditions (the more abundant light could influence the larval development of lycaenid butterflies; see e.g. Høegh-Guldberg 1968), and/or (3) the more balanced diet the *Myrmica* colonies received – thus, my diet seems to be suitable for *Myrmica* colonies as supplementary food.

Elmes & Thomas (pers. comm.) recorded similarly short times of development for *M. rebeli* from the Pyrenees and the Southern Alps under unnaturally warm conditions and with abundant food. Hence, an accelerated development is known from several populations and is not a unique phenomenon. These results support the plasticity of the developmental time of *M. rebeli* as the larvae develop during one or two years in nature (Elmes et al. 2001; Schönrogge et al. 2000; Thomas et al. 1998) or have a conspicuous accelerated development within one year under favourable conditions. In addition, based on my own observations, there is no indication of a two-year development of *M. rebeli* in Hungary since I have never found semi-developed *M. rebeli* larvae in *Myrmica* nests during the flying period. On the other hand, some semi-developed *M. alcon* larvae were observed in various Hungarian sites during the flying period. According to Varga (pers. comm.), 'dwarf' adults of *M. rebeli* regularly appear in several Hungarian populations at the end of the flying period (end of June to mid-July, depending on year and elevation). However, in the laboratory, the two dwarf specimens eclosed in late August. This suggests that undernourished *M. rebeli* larvae may also fully develop within one year under natural conditions and a partly bivoltine life cycle in nature seems to be unlikely. It is known that the growth of the one-year *M. rebeli* larvae tend to be fast immediately after adoption, stops during winter (meaning that they go into diapause in nature), and resumes in the spring just before pupation (Thomas et al. 1998). However, according to my laboratory observations the development of *M. rebeli* larvae can be continuous (without diapause) under favourable conditions, contrary to that of *M. alcon*. These differences were also observed when I reared *M. alcon* and *M. rebeli* larvae under the same laboratory conditions but in *Manica rubida* (Latreille, 1802) colonies (Tartally 2004). Further studies are still necessary to investigate the temporal dynamics of the development within *M. rebeli* and *M. alcon* populations and to understand the ecological circumstances influencing these dynamics.

Acknowledgements

I would like to thank Dr. David R. Nash, Enikő Tóth, Dr. Graham W. Elmes, Dr. Jeremy A. Thomas, Péter Kozma, Sándor Csősz, Dr. Sándor Szabó, Dr. Zoltán S. Varga and the referees for their help and for their critical comments. I very much appreciate the careful editing of the manuscript. Research has been funded by the EC within the RTD project "MacMan" (EVK2-CT-2001-00126).

References

Als, T. D., D. R. Nash & J. J. Boomsma 2001. Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycenidae) by three *Myrmica* ant species. – Animal Behaviour **62**: 99–106.

Als, T. D., R. Vila, N. P. Kandul, D. R. Nash, S.-H. Yen, Y.-F. Hsu, A. A. Mignault, J. J. Boomsma & N. E. Pierce 2004. The evolution of alternative parasitic life histories in Large Blue butterflies. – Nature **432**: 386–390.

Bereczki, J., K. Pecsenye, L. Peregovits & Z. Varga (in press). Pattern of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. – Journal of Zoological Systematics and Evolutionary Research.

Bhatkar, A. P., & W. H. Whitcomb 1970. Artificial diet for rearing various species of ants. – Florida Entomologist **53**: 229–232.

Buschinger, A. & E. Pfeifer 1988. Effects of nutrition on brood production and slavery in ants (Hymenoptera, Formicidae). – Insectes Sociaux **35**: 61–69.

Elfferich, N.W. 1988. Zuchterfahrungen mit *Maculinea alcon* (Denis & Schiffermüller, 1775) (Lepidoptera, Lycaenidae). – Mitteilengen der Entomologischen Gesellschaft Basel **38**: 134–150.

Elmes, G. W., J. A. Thomas, M. L. Munguira & K. Fiedler 2001. Larvae of Lycaenid butterflies that parasitize ant colonies provide exceptions to normal insect growth rules. – Biological Journal of the Linnean Society **73**: 259–278.

Elmes, G. W., J. C. Wardlaw, K. Schönrogge & J. A. Thomas 2004. Food stress causes differential survival of socially parasitic larvae of *Maculinea rebeli* (Lepidoptera, Lycaenidae) integrated in colonies of host and non-host *Myrmica* species (Hymenoptera, Formicidae). – Entomologia Experimentalis et Applicata **110**: 53–63

Høegh-Guldberg, O. 1968. Evolutionary Trends in the Genus *Aricia* (Lep.). *Aricia* Studies No. 9. – Natura Jutlandica No. 14, Aarhus, Denmark, 77 pp.

Hölldobler, B. & E. O. Wilson 1990. The Ants. – Springer Verlag, Berlin, 732 pp.

Kolev, Z. 2002. The species of *Maculinea* van Eecke, 1915 in Bulgaria: distribution, state of knowledge and conservation status (Lycaenidae). – Nota lepidopterologica **25**: 177–190.

Munguira, M. L. & J. Martin (eds.) 1999. Action Plan for the *Maculinea* butterflies in Europe. – Nature and Environment, No 97. Council of Europe Publishing, Strasbourg. 64 pp.

Pech, P., Z. Fric, M. Konvička & J. Zravý 2004. Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily. – Cladistics **20**: 362–375.

Schönrogge, K., J. C. Wardlaw, J. A. Thomas & G. W. Elmes 2000. Polymorphic growth rates in myrmecophilous insects. – Proceedings of the Royal Society of London, Series B **267**: 771–777.

Schönrogge, K., J. C Wardlaw, A. J. Peters, S. Everett, J. A. Thomas & G. W. Elmes 2004. Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. – Journal of Chemical Ecology **30**: 91–107.

Sielezniew, M. & A. Stankiewicz 2004. *Gentiana cruciata* as an additional host plant of *Maculinea alcon* on a site in eastern Poland (Lycaenidae) – Nota lepidopterologica **27**: 91–93.

Steiner F., M. Sielezniew, B. C. Schlick-Steiner, H. Höttinger, A. Stankiewicz & A. Górnicki 2003. Host specificity revisited: New data on *Myrmica* host ants of the Lycaenid butterfly *Maculinea rebeli*. – Journal of Insect Conservation **7**: 1–6.

Tartally, A. 2004. Is *Manica rubida* (Hymenoptera: Formicidae) a potential host of the *Maculinea alcon* (Lepidoptera: Lycaenidae) group? – Myrmecologische Nachrichten **6**: 23–27.

Tartally, A. & S. Csősz 2004. Adatok a magyarországi *Maculinea* fajok (Lepidoptera: Lycaenidae) hangyagazdáiról. (Data on the ant hosts of the *Maculinea* butterflies (Lepidoptera: Lycaenidae) of Hungary.) – Természetvédelmi Közlemények **11:** 309–317.

Thomas, J. A. 1995. The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. **13**: 180–196. – *In*: Pullin, A. & H. Chapman (eds.), Ecology and conservation of butterflies. – Chapter, London

Thomas, J. A., & G. W. Elmes 1993. Specialized searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. – Animal Behavior **45**: 593–602.

Thomas, J. A., & G. W. Elmes 2001. Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. – Proceedings of the Royal Society of London, Series B **268**: 471–477.

Thomas, J. A., G. W. Elmes & J. C. Wardlaw 1993. Contest competition among *Maculinea rebeli* butterfly larvae in ant nests. – Ecological Entomology **18**: 73–76.

Thomas, J. A., G. W. Elmes & J. C. Wardlaw 1998. Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. – Proceedings of the Royal Society of London, Series B **265**: 1895–1901.

Thomas, J. A., G. W. Elmes, J. C. Wardlaw & M. Woyciechowski 1989. Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. – Oecologia **79**: 452–457.

Thomas, J. A. & J. C. Wardlaw 1992. The capacity of *Myrmica* ant nest to support a predacious species of *Maculinea* butterfly. – Oecologia **91**: 101–109.

Wardlaw, J. C. 1991. Techniques for rearing *Myrmica* ants (Hym.) and *Maculinea rebeli* Hir. caterpillars (Lep., Lycaenidae). – Entomologist's Monthly Magazine **127**: 233–241.

Wardlaw, J. C., G. W. Elmes & J. A. Thomas 1998. Techniques for studying *Maculinea* butterflies: I. Rearing *Maculinea* caterpillars with *Myrmica* ants in the laboratory. – Journal of Insect Conservation **2**: 79–84.

Wardlaw, J. C., J. A. Thomas & G. W. Elmes 2000. Do *Maculinea rebeli* caterpillars provide vestigial mutuality benefits to ants when living as social parasites inside *Myrmica* ant nests? – Entomologia Experimentalis et Applicata **95**: 97–103.

Appendix

The recipe of the dry diet

Ingredients. 100 cm^3 (=14–15 g) freeze-dried fish [1]
1 level tablespoon of flour
1 pinch of sea salt
1 vitamin pill [2]
1 egg

[1] You can buy it in well-equipped pet shops as food for cats and turtles (I used a Hungarian product: Bio-Lio). If you cannot find freeze-dried fish you can dry some lean pieces of cooked fish or chicken in the sun or under an infra-red lamp.

[2] Choose the type that contains the daily portion of multiple vitamins, essential minerals, and salts for an adult (I used Supradyn).

Preparation. Grind the fish into powder and mix it with the flour, the salt, and the pulverised pill. Beat the egg slightly and add a little to the dry components. You need to get a hard paste in order to be able to form a ball, then leave it to desiccate for about half an hour. When its consistency is suitable, grate it with a cheese grater onto a sheet of paper. You will get various sizes of granules. Spread the granules on the paper and leave them to dry for about a day. The dried granules keep their quality (= the ants like them) for about half a year at room temperature in a dark and ventilated place.

I have planned this diet to culture *Myrmica* colonies because it is essential for the *Myrmica* colonies to be fed with protein and sugar (Wardlaw et al. 1998). However, the Bhatkar diet (Bhatkar & Whitcomb 1970) – which is presumably the most popular artificial ant diet (see e.g. Hölldobler & Wilson 1990) – contains very little protein (Buschinger & Pfeifer 1988).